REMARKS

Claim 1 and claim 63 have been amended to more particularly claim the present invention. Currently claims 1-8, 10, 12, 14-22, and 63 are pending in the present application.

Removal of Wong and Mammarella References

Applicants appreciate the removal of the Wong and Mammarella references that were not prior art. Applicants also herewith submit that since all six combinations of different prior art all utilized the Wong and Mammarella references, the Office Actions dated 3/3/09 and 5/11/09 were not valid. By removing Wong and Mammarella, none of the previous rejections and combinations included each and every element of the claims, and as such the application should have been allowed instead of continuing the injustice of issue yet again another office action where none of the cited references, alone or in combination teach each and every element of the claims. This prosecution has been proceeding for over seven years and applicants respectfully submit that since the Examiner has not identified in all this time references that anticipate or render the invention obvious, it is time to acquiesce and allow this case.

Claim Rejections – 35 U.S.C. § 112

The examiner has rejected claim 69 under 35 U.S.C. § 112, second paragraph as being indefinite. Applicants respectfully submit that the Examiner has made an error in not taking into account the knowledge and understanding of one skilled in the art in the field of liposome preparations and the study of liposome pharmacokinetics. Claim 69 is not indefinite to a person skilled in the art who is well conversant with the terminology of circulation time of a therapeutic or diagnostic agent (active material). In studying pharmacokinetics, the circulation time of the therapeutic or diagnostic agent (active material) is measured upon administration of a solution or an emulsion as this is a normal delivery method of the therapeutic or diagnostic agent (active material). When the therapeutic or diagnostic agent (active material) is encapsulated or coated and delivered in the same way, and the circulation time of the therapeutic or diagnostic agent (active material) is measured, any increase in that circulation time is attributed to that coating or the encapsulation. Likewise, if the diagnostic agent (active material) is in a liposome, the

circulation time of the therapeutic or diagnostic agent (active material) in the liposome is attributed to the liposome in which it is encapsulated. This is simply because the therapeutic or diagnostic agent (active material) would remain in circulation until it is in the liposome. Even if the active material is slowly leaking through the liposome and even if the therapeutic or diagnostic agent (active material) is exhausted, the liposome can still remain in circulation. Therefore, these measurements always show that liposomes are capable of holding the therapeutic or diagnostic agent (active material) for such long periods. It is but natural that unless the liposome remains in circulation, the therapeutic or diagnostic agent (active material) would not show such circulation time. Therefore, the meaning of the wording of claim 69 is clear to one of ordinary skill in the art. The purpose of preparing a liposome is to entrap some material in it. There is no purpose to inject a blank liposome in a human system, and no purpose in studying its circulation time. The liposome stability in the presence of the entrapped therapeutic or diagnostic agent (active material) is the most relevant use of the liposome. The whole purpose of using the liposome is to keep the therapeutic or diagnostic agent (active material) in circulation for a certain time. Therefore, if one has to find out how much the circulation time of a liposome has increased, one has to compare it with the circulation time of the therapeutic or diagnostic agent (active material) itself as it is normally injected (which is what is referred to as a "non-liposomal therapeutic or diagnostic agent (active material)." That is the basic standard by which measurements of liposome stability are measured. There is nothing like a standard liposome, and as such, the industry does not compare circulations times to some standardized liposome (that doesn't exist) but rather to the therapeutic or diagnostic agent administered alone. There is no standardized liposome (non-pegylated or pegylated liposome), in part because liposome properties vary according to their contents and their method of preparation.

The findings of the present invention show that the circulation time of the liposomal therapeutic or diagnostic agent (active material) - that is entrapped in the liposome of the present invention, is increased more than 25 times than that of circulation time of the non-liposomal therapeutic or diagnostic agent (active material) in the form as commonly used at the time of invention. One skilled in the art would understand that this is a normal comparison and would clearly understand the metes and bounds of the invention and what the applicants have defined as their invention.

The question is why is this property of the liposome claimed -- because this property of the liposome defines its structural difference from other liposomes of similar compositions and/or size. The inventors believe that it is only the structure of the liposome that makes it hold the therapeutic or diagnostic agent (active material) entrapped in it for longer time. Prior to the present invention, others altered the structure by adding polyethylene glycol (PEG). Pegylated liposomes, because of the polymer coatings on the bilayers of the liposome membranes, have been shown to hold the therapeutic or diagnostic agent (active material) for longer time than non-pegylated liposomes. In contrast, the present invention has altered the structure of the liposome by its method of manufacture and not the addition of PEG. As such, there is no way to show such structural difference here as compared to other non-pegylated liposomes, nor is it required to give any reason for such performance or any evidence for such, in a patent specification. When the structure and function is so closely related, the function expresses the structure. This is similar to the concept when dimensions of particles of powder cannot be measured, one describes them by saying it passes through a certain size mesh, for example.

Provided below is a more clarification on this terminology and the methods of measurements of circulation time. Table 1 in the specification of the present invention is reproduced below:

TABLE 1

ACUTE TOXICITY AND PHARMACOKINETIC STUDIES IN MICE

Parameters	Example II	CAELYX	ADRIAMYCIN
LD_{50} (mg/kg)	16.13	13.5	10.29
MTD (mg/kg)	8	8	5
C_{max} (µg/ml)	267.54	265.34	26.8
T_{max} (hours)	0.0385	0.085	0.085
Kel	0.08697	0.07289	4.851831
$T_{1/2}$ (hours)	6.946	9.388	0.143
AUC (µg-l/ml)	1698.024	2663.215	1.244
Vd (ml)	1.489	1.858	61.42
Vd (ml/kg)	59.58	67.52	1656.79
Cl (ml/h)	0.15	0.12	200.96

Abbreviations:
MTD = maximum tolerated dose;
C_{max} = maximum concentration of drug achieved in the plasma;
T_{max} = time taken to achieve the maximum concentration of drug in the plasma;
Kel = elimination constant;
$T_{1/2}$ = time required for the drug concentration in the plasma to get decreased by 50%;
AUC = area under "concentration" vs. "time"curve;
Vd = volume of distribution;
Cl = clearance rate of drug

From Table 1 in the specification, it is seen that the time taken to achieve the maximum concentration (T_{max}) of doxorubicin (ADRIAMYCIN) is the same for free doxorubicin and for

the liposomes of the present invention. Table 1 also shows that the amount of Doxorubicin (ADRIAMYCIN) in the plasma, at the maximum concentration (C_{max}) when a composition of the present invention is injected, is 10 times more than that in the conventional non-liposomal composition. In addition, the time required for the Doxorubicin (ADRIAMYCIN) concentration in the plasma to get decreased by 50 % (which is referred as $T_{1/2}$) is almost 50 times [6.948/ 0.143 = 48.587] that required for conventional non-liposomal composition. If we take 1 unit for half circulation time ($T_{1/2}$) of the conventional non-liposomal composition, it means the liposomal composition of the present invention would be [48.5 -1 =] 47.5 units times longer than that of the conventional non-liposomal composition.

Example IV of the present specification describes the method of determining the concentration of drug (doxorubicin) in blood plasma. The cited reference, Hong et. al. (Clinical Cancer Research, vol.5, November 1999) on page 3646 in the left side column under sub-heading pharmacokinetics, shows a similar method. Consider other half circulation times for other preparations as reported by Hong, or other publications. For example in Hong, there is published data for $T_{1/2}$ for pegylated and non-pegylated liposomes. In Table 1 of Hong, it shows that for 0% PEG, the circulation time is 12.5 hours. With 6% PEG the circulation time is 25.1 hours. The maximum circulation time obtained with pegylated liposomes is obtained with 6% PEG. Thus, the circulation time of the present liposomes are twice that of non-pegylated liposome, when the non-pegylated as well as the pegylated liposomes is made by the HONG process. In the instant invention, considering the data from Table 1, $T_{1/2}$ of pegylated liposome Caelyx is 9.7/6.9 = 1.4 times that of the instant invention. Or in other words, the liposomes of the present invention show a circulation time that is 0.71 of the pegylated liposome (or 71 %) that of the pegylated liposome. This comparison is not considering a comparison with ADRIAMYCIN.

The Examiner has raised a further point that it is natural that a liposomal formulation will have longer circulation time than the non-liposomal formulation. Yes, the point is how much longer? The maximum increase of circulation time of non-pegylated liposomes is limited and to increase this length of time, others pegylated the liposomes. In contrast to pegylating the liposomes to obtain a longer circulation time, the present inventors utilized a novel method of manufacture for non-pegylated liposomes. The non-pegylated liposomes of the present invention

have achieved an increased circulation time close to the circulation time achieved by pegylated liposomes (~71% as shown in the preceding paragraph).

The above discussion should reply to the Examiner's question of, "what is being tested?" Liposomal therapeutic or diagnostic agent (active material) is being tested against non-liposomal therapeutic or diagnostic agent (active material)(which is the standard comparison). This test results in the measurement of the effect of the liposome and therefore what is being tested is the intact liposome of the present invention and not merely its component phospholipid/cholesterol mixture.

Further, in response to the Examiner's rejection, one skilled in the art would understand the term "ADRIAMYCIN" especially since the ADRIAMYCIN composition has been described on page 8 of the specification, which is reproduced below for quick reference.

> **[0101]** CAELYX (Pegylated liposomal Doxorubicin formulation) manufactured by Ben Venue Laboratories, USA and ADRIAMYCIN (Conventional non-liposomal Doxorubicin formulation) manufactured by Pharmacia & Upjohn, USA were used in animal studies for comparative evaluation with Non-pegylated liposomal Doxorubicin formulations of the present invention. ADRIAMYCIN, which is also referred to herein as "Conventional non-liposomal doxorubicin composition" is a freeze dried sterile powder for injection, each vial containing Doxorubicin hydrochloride 10 mg, Lactose 50 mg, Methylhydroxybenzoate 1 mg. Before use, the freeze dried powder is reconstituted with 5 ml of Water for Injection provided with the pack.

Further, in response to the Examiner's rejection that the expression "wherein the non-pegylated doxorubicin liposomes have a circulation time in blood at least 25 times longer than that obtained with ADRIAMCYIN when tested in Swiss albino rats" is unclear, applicants respectfully submit that one skilled in the art would clearly understand that this is about testing liposomes of the present invention with Doxorubicin entrapped in them, and comparing them with ADRIAMYCIN (which is described in the specification as a commercially available non-liposomal doxorubicin composition). Claim 1 does not refer to liposome loaded with doxorubicin, but the liposomes as prepared by the present invention (such as in Claim 1) are tested with doxorubicin in them. Here, the role of doxorubicin is as the therapeutic or diagnostic agent (active material) used for testing, $T_{1/2}$ There is no need for claim 1 to recite any active ingredient. It defines liposomes as a carrier for suitable therapeutic or diagnostic agents (active materials), etc. With the understanding that for the testing purpose, any therapeutic or diagnostic agent (active material) could have been used, but in the present invention doxorubicin has been

used. In fact, the liposomes with the loaded therapeutic or diagnostic agent (active material) are another embodiment of the invention.

It is clear from the paragraph from instant specification quoted above (Para [0101]) in that ADRIAMYCIN powder is reconstituted with 5 ml of water for injection provided with the powder as recommended for clinical administration by the manufacturer. Therefore it is the best standard for comparison, away from variations arising, if it is used in any other form, as thought by examiner. The Examiner's argument that ADRIAMYCIN is "a solid and one can make different forms of non-liposomal compositions such as emulsions..." shows that the Examiner has ignored the teaching of the specification, which clearly shows how the ADRIAMCYIN was prepared for the testing.

Accordingly, applicants submit that claim 69 is not indefinite and request withdrawal of this ground of rejection.

Claim Rejections – 35 U.S.C. § 112

I. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Hong (Clinical Cancer Research, 1999), Janoff (US 4,880,635) and Papahadjopoulos (US 4,235,871), optionally in further combination with Barenholz (US 5,316,771).

A. Hong does not teach each and every element of the claimed invention, or cures the deficiencies in the other cited references.

Hong is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. As discussed in previous responses to office actions and in the Appeal Brief, Hong does not teach or suggest at least two elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; and 2) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

First, Hong teaches using pegylated liposomes to prolong liposome circulation. Thus, Hong's liposomes results in the problems that the instant inventions solves by not using pegylated liposomes, such as Hand and Foot Syndrome.

Additionally, there is no teaching in Hong that would have led one of skill in the art to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for

hydrating phospholipid would reduce leakage from a loaded liposome. Further, there is no evidence to say that leakage of liposomes has any relation with long circulation of liposomes, as required by the claims.

Hong's experiment as described employs DSPC: Cholesterol at a 3:2 molar ratio; with variable PEG-DSPE. The contents were hydrated at 55 °C in ammonium sulphate solution (250 mM at pH 5.0) and extruded through polycarbonate membrane filters of 0.1 to 0.05 μm pore size. The elements in claim 1 of the instant invention that are not taught or suggested by Hong are reproduced below:

(1) removing the solvent or mixture of solvents and adding an aqueous hydration media to the phospholipids and sterols; or

(2) adding an aqueous hydration media to the phospholipids and sterols in the solution; and removing the solvent or mixture of solvents;

(3) removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution.

(4) wherein the aqueous hydration media comprises consists essentially of ammonium sulfate and sucrose and

(5) the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes;

In addition, Hong demonstrates that there is not a "standard" pegylated liposome. The pharmaceutical properties of pegylated or non-pegylated liposomes vary from preparation to preparation. Amongst the pegylated liposomes, the extent of pegylation, the molecular weight of PEG used, and the manner of pegylation make a difference.

The purpose behind the Examiner's statement that "According to Hong, paradoxically, the group of mice treated with liposomal doxorubicin without PEG had higher tumor doxorubicin concentrations" (Abstract) is unclear and appears to be a red herring. It is known by those skilled in the art that: "The tumor accumulation of liposomes is not always correlated with their circulation time in the blood." See Uchiyama (Abstract)(International Journal of Pharmaceutics, 1995)(cited art in previous rejections). Uchiyama also noted that the "accumulation of liposomes from the vascular space into the tumor is primarily governed by their size and not by their membrane fluidity or blood circulation time." The instant invention is concerned with blood circulation time.

In the Hong process, it is clear that there is no sucrose or buffer stated to be present in the hydration medium. So one difference from claim 1 of instant invention is: Hong contains no sucrose in the phospholipid hydration medium. It is unclear the Examiner's intentions in his statement "Although pH of these solutions is indicated, it is unclear whether buffers are used to prepare the ammonium sulphate solution." If the Examiner is implying that buffers are present in Hong, then this adds another difference from the instant invention as claim 1 does not have any buffer in the hydration medium (as indicated by the presently amended claims "wherein the aqueous hydration media ~~comprises~~ consists essentially of ammonium sulfate and sucrose. . . .")

It appears that the examiner has combined the two solutions used in the present invention together when he says "these solutions." However, it is clear by the claim language that in the present claim 1, there are two different and distinct solutions, each used for a different purpose: one is a hydration medium and the other is a sucrose-histidine buffer solution for removing extra liposomal ammonium sulphate. As discussed above, Hong does not teach or suggest the present claimed hydration medium, nor does Hong teach or suggest the step of removing extra-liposomal ammonium sulphate in the process.

What is not expressed and considered as implicit is the Examiner's own thinking based on hindsight. How can one imagine that the step of removing extra liposomal ammonium sulphate is by using dialysis with sucrose-histidine buffer solution, when the entire process described by Hong on page 3646 does not have the words "sucrose" or "histidine," let alone the combination of a "sucrose-histidine buffer". One of ordinary skill in art would not think of such specific materials or actions, and therefore the Examiner's conclusions are outside the spirit of the obviousness concept. It is necessary to state facts related to the process of making liposomes along with its properties because the structure of the liposome depends on the process of making it and its properties are dependent on its structure. With due respect to the Examiner, it is not persuasive to separate the two, and say that even if some part of the process is different the properties would remain the same as this is not how the manufacture of liposomes works.

It should be noted that the instant invention does not also use a sucrose-histidine buffer as a hydration medium. Hong uses only ammonium sulphate solution for hydration whereas the present invention uses an aqueous hydration media that consists essentially of ammonium sulfate and sucrose. Further, the present invention requires a specified amount of hydration solution ("in the range of 10 to 35 ml for each mmole of phospholipid") in contrast to Hong, who has not

described the amount of hydration solution as that was not of any importance to him, and was never thought that the quantity of hydration buffer had any role to play in the structure/property of the liposome produced. After extensive experimentation, in the instant invention, it was found that when hydration medium is employed at a limited level, the liposomes formed have some structure that gives a longer circulation than when the hydration is performed with larger amounts of the hydration media. There is no state of art at the time of the instant invention to teach or suggest that one skilled in the art would know that the liposome formed will have a different property if the hydration medium amount is changed. Therefore, just knowing how much hydration buffer is needed to complete the formation of liposome from the phospholipids taken is not relevant. The hydration amount used in the instant invention is not related to the sufficiency or insufficiency of liposome formation. The role of the quantity of hydration medium was explored in the instant invention to result in a synergistic property of increasing circulation time of the liposome prepared by the entire process.

Hong cannot be combined with Papahadjopoulos because the very purpose of the Examiner's combination with Papahadjopoulos (to show that it uses the same volume of hydration media as in the instant invention), has been shown to be incorrect (see discussion below). In addition, there is no teaching in Hong that would have led the skilled reader to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Further, there is no evidence to say that leakage of liposomes has any relation with long circulation of liposomes, as required by the claims.

B. Papahadjopoulos does not teach or suggest each and every element of the claimed invention.

Papahadjopoulos does not teach or suggest each and every element of the claimed invention. This reference has been previously discussed in responses to other office actions and in the Appeal Brief.

On page 5 of the Office Action dated October 6, 2010, the Examiner erroneously concludes that:

> Papahadjopoulos discloses methods of formation of liposomes. The methods involve either removal of the organic solvent before hydration (Example 1) or making an emulsion using an organic solvent containing phospholipid and an

aqueous medium and evaporating the organic solvent (Example 2). In either method, the amount of lipid is 100 micromoles and the aqueous medium added is 1.5 ml which corresponds to 15 ml of aqueous medium per millimole of the phospholipid.

Applicant's observations on Papahadjopoulos are as follows. As noted in the summary of the Papahadjopoulos invention, the "invention comprises a method of encapsulating biologically active materials in synthetic, oligolamellar lipid vesicles."

Examples 1 and 2 in Papahadjopoulos are presented in tabular format to help understanding the steps in these examples.

Process steps of Papahadjopoulos	**Applicant's Observations and Comparison to Present invention**
[Phosphatidylglycerol + phosphatidylcholine + cholesterol 10, 40 & 50 µM respectively] dissolved in chloroform, the solvent is evaporated.	Phospholipid contains phosphatidyl glycerol; Initial solvent amount is not given
The thin lipid layer in the flask is then purged with nitrogen and 5 ml of diethyl ether added with stirring to dissolve the lipids.	The 2nd solvent added is 5 ml and remains in the organic phase when the hydration media is added.
Then 1.5 ml aqueous buffer - pH of 7.4 [10 mM sodium chloride /4 mM of histidine/"TES"(2{[tris(hydroxymethyl)methyl] amino{ethanesulfonic acid] and 10 mg/ml of alkaline phosphatase* is added to form a heterogeneous, 2-phase mixture. The mixture is emulsified by sonication.	The hydration media contains histidine, which is not used in instant invention at the hydration stage. No ammonium sulphate or sucrose is present in the hydration buffer (as required by the present invention). The buffer also includes *the biologically active material to be entrapped (i.e. alkaline phosphatase or RNA).
The resulting emulsification is evaporated at 25 °C**/10-50 mm Hg until a viscous gel results. Then 1.5 ml. of the sodium chloride/histidine/TES buffer described above is added to the gel to obtain an aqueous suspension of the gel. The resulting mixture is evaporated at 30 °C/ 10-50 mm Hg / 15 minutes to obtain an opaque suspension of phospholipid vesicles (synthetic liposomes) 0.2 to 0.6µ.	This formation of the gel, diluting with buffer and again evaporating are not present in the present invention. Further, the two-stage addition of aqueous phase is not in the instant invention.

* In Example 2 - 1 mg/ml of ribonucleic acid (RNA) (either polyadenylic acid or 25S tetrahymena ribosomal RNA) instead of alkaline phosphatase.
**In Example 2 – it is 0°C

1. Papahadjopoulos does not teach or suggest "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present."

Calculation of volume of hydration buffer per mM of lipid in Example 1 and in Example 2

The amount of phospholipid used is 50 μM (cholesterol is 50 μM) and there is 5ml ether in organic phase. In addition, there is 1.5 ml aqueous phase buffer plus 1 ml (– 10mg) from the alkaline phosphatase solution. Although it is not precisely clear how much water of this aqueous phase remains in viscous gel, applicants assume that the maximum is 2.5 ml. In addition, another 1.5 ml of aqueous phase buffer is added to the gel when it changes to liposomes. At that stage the maximum water content would be 4 ml. Thus, 4 ml of aqueous phase for 50 μM converts to 4,000 per 50 mM, or 80 ml per mM, which is clearly outside of the range of the claimed invention. Even if one were to guess that there would be some loss of water during intermediate evaporation, even if assuming that the aqueous phase may be less than 4 ml (such as 3ml), there would be 3 ml of aqueous phase per 50 μM of phospholipid. This converts to 3000 ml per 50 mM; or 60 ml per mM. This is certainly outside the range of the instant invention Even if one calculated that only 2.5 ml aqueous phase remained, it would still be 2500 ml per 50 mM or 50 ml per mM and 2000 ml per 50 mM or 40 ml per mM respectively, which is above the range given in instant invention.

2. Papahadjopoulos does not teach or suggest removal of the solvent before hydration.

The Office Action's statement: "The methods involve either removal of the organic solvent before hydration (Example 1) or making an emulsion using an organic solvent containing phospholipid and an aqueous medium and evaporating the organic solvent (Example 2)" is incorrect. As shown above in the chart, as well as can be seen from reading the Papahadjopoulos patent, in Example 1 there is no removal of solvent before hydration, because the added solvent ethyl ether is present. There is no difference in Example 2 in this respect. Both examples follow the same procedure and the same quantities of the materials are used (the only difference being

the type and amount of the biologically active material used). The aqueous level is the same in Example 1 and 2. In other words, Papahadjopoulos does not teach a method of removing all solvent before hydration, to make lipid as of a dry film type before hydration, as in the instant invention. To the contrary, Papahadjopoulos needs the solvent to make an emulsion of water-in-oil when the buffer is added. Therefore, there is no teaching or suggestion in Papahadjopoulos whatsoever that the solvent should be removed before adding hydration medium.

3. Papahadjopoulos relates to entrapment quality and has no teaching regarding long-circulating liposomes, let alone even teach or suggest the hydration media in the claimed invention.

Papahadjopoulos correlates ionic strength of buffer with the entrapment quantity of biological material: encapsulation efficiency relates to the ratio of organic phase to aqueous phase and concentration of lipid/phospholipid in the two phase system. However, it neither measures nor correlates any parameter to the increase in circulation time of the liposome prepared and in fact has no such study on circulation time of the liposomes. Just because it was erroneously believed that Papahadjopoulos has a similar ratio of aqueous phase per phospholipid, it does not mean that it is obvious to one of ordinary skill to think that this ratio will increase circulation time of liposome. It is not possible to predict what process steps give rise to longer-circulation time that results in a greater efficiency in reduction in breast tumor and hence is not obvious to choose a particular ratio of aqueous phase to phospholipid from one reference for use in another process having altogether different process conditions. Therefore, it is unlikely to motivate one skilled in the art to think of applying a particular range of volume of aqueous hydration medium to the method of the present invention, which is different from that of Papahadjopoulos. In Papahadjopoulos examples 1 and 2, the hydration buffer is sodium chloride/histidine/TES buffer, whereas in the instant invention, the hydration buffer is ammonium sulphate/sucrose. It is not possible to predict what process steps will produce liposomes that would give rise to longer circulation time and hence greater efficiency in reduction in breast tumor growth. Therefore, it is not correct to say it is obvious to choose a particular volume of hydration medium of a different composition from that used in another process having altogether different processing conditions to get liposomes having pharmacological properties not reported or referred to, or suggested in that process.

4. Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase.

Since the instant invention also teaches a method wherein the solvent is removed before (or after) the addition of aqueous phase, (both of which give the same results), it is not persuasive to say that the step of removing the solvent before the addition of the aqueous phase is obvious from Papahadjopoulos. It should be noted that in Example 1 of Papahadjopoulos, one solvent is removed but another is added to dissolve the lipids, and the aqueous phase is added to the solution. Papahadjopoulos does not teach the removal of the solvent before the addition of the aqueous phase as argued by the Office Action.

Applicants note that the drug to be encapsulated is incorporated in the aqueous phase in Papahadjopoulos' procedure, whereas in the instant invention the empty liposomes are first prepared and the drug is loaded afterwards. The procedures are thus quite different and therefore it would not be obvious to one of ordinary skill in the art to think of using the step of removing the solvent after the liposomes are formed in the process for making non-pegylated long-circulating liposomes without any drug loaded in it.

5. Papahadjopoulos does not teach or suggest the use of ammonium sulphate and sucrose as a hydration buffer.

Papahadjopoulos does not teach or suggest use of ammonium sulphate and sucrose as a hydration buffer and further the buffers are internal to the liposomes and are not used to remove extra-liposomal hydration salt, for which the instant invention uses a histidine sucrose buffer.

Papahadjopoulos discloses the preparation of liposomes by emulsifying a mixture of lipids in organic solvent and an aqueous mixture of the active material for encapsulation; and then removing the organic solvent and suspending the resultant gel in water. Sucrose is included amongst exemplified active materials for encapsulation (see column 6, lines 31-43) and in the hydration buffer (column 10, lines 53-56) but there is no reference to ammonium sulfate. Thus, there is no teaching of a hydration buffer comprising ammonium sulfate and sucrose.

Papahadjopoulos' method does not say that liposomes can also be made by first removing the organic solvent and then hydrating the phospholipids. It is not obvious to think that it will work that way also.

In Papahadjopoulos, the hydration media is different, the drug is different, and the method of drug loading is different. In a broad sense, Papahadjopoulos' method of invention calls for the formation of "inverted micelles" in an organic phase and then the removal of organic phase. The system then spontaneously reverts to a bilayer-like structure, with a large amount of aqueous phase encapsulated in large oligolamellar vesicles. Accordingly, the methods of the present invention and Papahadjopoulos are not even similar.

6. Papahadjopoulos does not teach or suggest other numerous claim elements.

Examples 1 and 2 of Papahadjopoulos employ Phosphatidyl glycerol, the use of which is not indicated or claimed in the instant invention. In the instant invention the long circulation effect is achieved without the use of phosphatidyl glycerol; and therefore, the procedure of Examples 1 or 2 is not relevant to one of ordinary skill in the art to think that a particular range of ratio of aqueous phase to organic phase would lead to long circulating liposome having greater efficiency in reducing breast tumour, without the use of phosphatidyl glycerol.

In Papahadjopoulos, a buffer can be added during the liposome formation (see column 5, lines 30-35 & column 6, lines 5-13) and exemplified buffers include sodium chloride/histidine/2-{[tris(hydroxymethyl)methyl]amino} ethanesulfonic acid (TES) (see Examples 1, 2, 5, & 6). Histidine/TES buffer has been used in Examples 1 and 2 aqueous phase. There is no use of such buffer in the hydration medium in the instant invention.

C. Janoff does not teach or suggest each and every element of the claimed invention.

Janoff has been discussed in previous responses to Office Actions and in the Appeal Brief. Janoff is deficient in its asserted teachings and uncured by the remaining references for at least the following reasons. Janoff does not teach or suggest the hydration media claimed in the present invention. Janoff does not teach or suggest at least two elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; and 2) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

The Office action dated October 6, 2010 on page 5 states:

> Janoff teaches that sugars such as sucrose when present both inside and outside would enable the liposomes to retain Adriamycin during dehydration and

rehydration (Example 1; col. 21, line 23 through col. 21 line 27). Janoff further teaches the hydration of 80 micromoles of lipid with 21 ml of buffer (25 ml per mmole).

Janoff discloses the preparation of dehydrated liposomes by drying liposome preparations under reduced pressure in the presence of one or more protective sugars. Exemplified sugars include sucrose. The dehydration is conducted under vacuum with or without prior freezing of the liposome preparation. There is no reference to any ammonium salt or to any sulfate. Reference is made to loading rehydrated liposomes using a concentration gradient created after rehydration. In the exemplified processes, 80 μmoles EPC was hydrated with 2 ml aqueous solution containing 150 mM sodium chloride, 20 mM HEPES and the respective sugar (see column 8, lines 40-63).

The fact that sucrose or other sugar inhibits leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from the rehydrated liposomes after loading with the required drug or other active material. Further, there is no evidence to say that leakage of liposomes has any relation with long-circulation of liposomes. The instant invention hydrates phospholipids to form liposomes, Janoff hydrates dehydrated liposomes. These are quite different things.

It is interesting to note that the Janoff patent was first published as a PCT 86/1103 on February 27, 1986. Subsequently Mayer filed a patent on March 5, 1987 (see WO8806442 (A1) 1988-09-07) and referred to Janoff noting that sucrose can be used for dehydration. Mayer did not think of using sucrose in a hydration medium for hydrating phospholipids in making liposomes. Thus such use was not obvious from Janoff. Even as on the date of filing the instant application, December 31, 2002 (more than 15 years after the Janoff publication), nobody has used sucrose in the medium for hydration of phospholipids. This fact clearly establishes that it is not obvious to think that sucrose in combination with ammonium sulphate can be used in the phospholipid hydration medium for obtaining leak-proof liposomes and long-circulating non-pegylated liposomes.

In Janoff, extrusion technique vesicles (ETVs) were prepared using a solute solution containing ADRIAMYCIN and 250 mM trehalose. The samples were dehydrated for 24 hours without prior freezing. The ADRIAMYCIN content of the initial sample and the rehydrated vesicles was determined as described in Example 1. As clearly demonstrated by this Example,

the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration.

The Examiner states that Janoff teaches addition of sucrose to the phospholipid hydration HEPES buffer while preparing vesicles for protection of liposome membranes during dehydration and rehydration. The effect of such sugar addition while preparing vesicles is performed with certain buffers. In Example 1, ETVs are used and the concluding lines (Col. 11, lines 31 – 38) are as follows:

> The results of these experiments are shown in Table 1. As shown therein, more than 90% of the drug is retained following dehydration and rehydration, i.e. the same levels as those achieved with $^{22}Na^{+}$ and ^{3}H-inulin. Moreover, the rate of leakage of ADRIAMYCIN from the rehydrated vesicles is comparable to the rate observed with vesicles which have not been dehydrated (see Bally, et al., (1985), Biochim. Biophys. Acta., 812:66).

Appellants respectfully point out there is no need for dehydration or rehydration of liposomes, which is the case in the instant invention, and accordingly there is no need to add sugar according to Janoff. Janoff does not suggest that sugar addition will increase the circulation time. *See* Col.11, lines 39 – 43 noting that: "As clearly demonstrated by this Example, the sugar trehalose is capable of protecting liposomes during dehydration and subsequent rehydration so that more than 90% of the material encapsulated within the liposomes is still retained therein after rehydration." This conclusion also says that trehalose offers protection during dehydration and rehydration to materials encapsulated within the liposome and has nothing to do with increasing circulation time. It does not say that when liposomes do not go through dehydration or rehydration, trehalose will offer protection or not.

Further the results of experiments in Example 7 of Janoff leads to the conclusion:

> As shown by these results, well over 80% of the internal contents of each of the three types of liposomes were retained after the dehydration/rehydration process without the use of any protective sugars. Moreover, adding trehalose to these types of liposomes somewhat decreased, rather than increased, the amount of internal contents retained in the liposomes after the dehydration/rehydration process.

Column 16, lines 37 – 44.

This paragraph actually shows that the trehalose decreased the amount of entrapment. Thus, it is not proper to say that by reading Janoff a person having ordinary skill in the art would think that addition of sugar to ammonium sulphate containing hydration medium when there is no hydration and dehydration of liposomes, would produce liposomes having a long-circulation time.

It should be remembered that all inventions are made from known things and facts. Just knowing that sugar is a material that protects liposomes during rehydration, does not make it obvious to make liposomes with long circulation time and increase efficiency of reducing breast tumor when it is loaded with doxorubicin. All other factors including the materials used, each and every step in the preparation of such liposome contributes to make the invention. It is the concerted effect of the total process that gives the unexpected results.

Janoff does not show the addition of sucrose/sugar to the hydration media for getting it inside the liposome, and there is no motivation for doing so by reading Janoff --its abstract clearly says it is for stabilizing liposomes during hydration and rehydration.

The Examiner has asserted that Janoff teaches the use of a hydration media at 25 ml per mM of phospholipid. What is the hydration media? Sodium chloride, HEPES and trehalose buffer; what is the phospholipid? Egg phosphatidyl choline. This reference to 25ml per mM is not relevant to the conditions in instant invention – the phospholipids are different and the hydration media is different.

In column 5, line 54-68, Janoff writes:

> So that the liposomes will survive the dehydration process without losing a substantial portion of their internal contents, it is important that one or more protective sugars be available to interact with the liposome membranes and keep them intact as the water in the system is removed. A variety of sugars can be used, including such sugars as trehalose, maltose, sucrose, glucose, lactose, and dextran. In general, disaccharide sugars have been found to work better than monosaccharide sugars, with the disaccharide sugars trehalose and sucrose being most effective. Other more complicated sugars can also be used. For example, aminoglycosides, including streptomycin and dihydrostreptomycin, have been found to protect liposomes during dehydration (emphasis added).

This paragraph says that sucrose interacts with liposome membrane and keeps them intact as the water in the system is removed, and the liposome will survive the dehydration process. In the instant invention, there is no stage of dehydration or rehydration of the liposome; there is only hydration of phospholipids to prepare liposomes. So this quotation is irrelevant. However, upon reading the entire paragraph it is clear that trehalose or sucrose is useful for protection in dehydration, which is not a step in the instant process. The question is -- does Janoff suggest the use of sucrose with ammonium sulphate for hydration of phospholipid to form liposome? No. He does not. In Col.8, lines 40 –43 where Janoff describes how vesicles ETV are prepared, it is clear that Janoff does not use sugar with ammonium sulphate during hydration of phospholipids for making liposomes. Rather Janoff is using sodium chloride, and HEPES buffer with sugar for protecting liposome structure during dehydration. Further, there is no reasoning or motivation to pick only sucrose from a laundry list of sugars in Janoff and combine it with ammonium sulfate to achieve a long lasting non-pegylated liposome when Janoff uses sodium choloride and HEPES buffer along with a sugar for a different purpose altogether. To reach the Examiner's conclusion, one would have to speculate in hindsight reasoning. It is not obvious to a person of ordinary skill in the art. The hydration of phospholipids with ammonium sulphate and sucrose to form liposomes is for the first time shown in the present invention.

D. Barenholz does not teach or suggest each and every element of the claimed invention.

Barenholz discloses transmembrane loading of amphipathic therapeutic or diagnostic agents (active material) into liposomes using an ammonium transmembrane gradient. In the exemplified process, a lipid film is hydrated with aqueous ammonium sulphate containing desferal (desferoxamine mesylate). In each case, 5 ml of the solution was added to a film formed from 100 mg egg phosphatidyl choline. In contrast, the presently claimed method requires an aqueous hydration media that consists essentially of ammonium sulfate and sucrose and the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present. Barenholz thus does not teach or suggest the use of sucrose in the ammonium sulphate hydration solution. Further, Barenholz's solution uses desferal, which the present invention does not (as the claim recites "consists essentially of ammonium sulfate and sucrose").

Further, Barenholz also does not teach or suggest another element of the claimed invention: "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution." None of the Examples in Barenholz show any dialysis method or spell out any dialysis buffer. These details are not obvious from Barenholz, as nowhere in Barenholz this has been taught or suggested.

In conclusion, none of the cited references, alone or in combination teach all of the recited elements of claim 1. The lack in the teaching of these references to derive a process of amended claim 1 is significantly more than the combined use of ammonium sulfate and sucrose and the amount of aqueous hydrating medium per mole phospholipid. As such, applicants request withdrawal of this ground of rejection.

II. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Slater (US 6,355,268) in view of Janoff (US 4,880,635).

A. Slater fails to teach or suggest all of the claimed elements, alone or in combination with Janoff (or any other cited art).

Slater fails to teach or suggest all of the claimed elements, alone or in combination with Janoff (or any other cited art). The present claim 1 recites the following steps:

a) removing the solvent or mixture of solvents and adding an aqueous hydration media to the phospholipids and sterols; or

b) adding an aqueous hydration media to the phospholipids and sterols in the solution; and removing the solvent or mixture of solvents;"

Of these two alternative elements of the claim 1, Slater has only the second alternative, he does not disclose the first alternative. Even for this second alternative Slater is not relevant because he needs pegylated phospholipid in phospholipids used for preparing the liposome, whereas the instant claim relates to non-pegylated liposomes. In addition, Slater shows these steps in reverse order: first doing step (b) - adding an aqueous hydration media to the phospholipids and sterols and then doing step (a) - removing the solvent or mixture of solvents. However, for this claim element, Slater is not relevant. Slater needs a pegylated phospholipid in the liposome, whereas the instant claim relates to non-pegylated liposomes. In the abstract, Slater clearly states - (liposomes) "being composed of a vesicle forming lipid and of a vesicle-forming lipid

derivatized with a hydrophilic polymer to form a coating of hydrophilic polymer chains on both the inner and outer surfaces of the liposomes." Further, on col. 2, lines 52 -64; and col. 3 lines 16 – 67 fully describes the pegylated phospholipids used in Slater. Pegylated liposomes are essential in Slater's liposomes. There are two Examples in Slater describing the preparation of liposomes (Example 1 and Example 4). In both these examples the pegylated liposomes have mPEG-DSPE as derivatized vesicle forming lipid. (See col. 20 line 39 and 23 line 16.)

In Slater, the ammonium sulphate and ethanol were removed from the external bulk aqueous phase immediately prior to remote loading of the active agent by hollow fiber tangential flow diafiltration. This step is clearly different than the claimed process. In the above step, the claimed process removes only the organic solvent, whereas the Slater process removes both ammonium sulphate and ethanol together by tangential flow diafiltration. In example 1 there is a step of removing extra liposomal ammonium sulphate along with ethanol. In example 4 there is no ammonium sulphate, so dextran sulphate is removed with sodium chloride solution followed by a sucrose solution (see lines 39 – 44 in col. 23 Example 4.). So, Slater does not teach or suggest the claim element of "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution."

In the instant claimed process, the therapeutic or diagnostic agent (active material) is taken in a sucrose-histidine buffer, whereas in Slater's process the therapeutic or diagnostic agent (active material) is taken in a sucrose solution.

In addition, Slater does not teach or suggest the claim element of an aqueous hydration media consisting essentially of ammonium sulphate and sucrose. In Example 1, col.20 line 47- there is a 250 mM ammonium sulphate solution; but no sucrose. In Example 4, col. 23 lines 27 – 29 there is a dextran sulphate solution – and thus, there is neither ammonium sulphate nor sucrose in this hydration medium. Thus, Slater does not teach an aqueous hydration buffer consisting essentially of ammonium sulphate and sucrose.

Slater does not teach or suggest the claim element of removing extra-liposomal hydration media using a sucrose-histidine buffer solution. (Example 1, col. 21 lines 2 - 9; Example 4, col.23 lines 39 – 44).

B. Janoff fails to teach or suggest all of the claimed elements, alone or in combination with Slater (or any other cited art).

The deficiencies of Janoff were discussed above. Additional comments are provided below. Janoff used a hydration buffer as described below in Col.8 lines 41-43:

> hydrated with 2 ml of 150 mM NaCl, 20 mM HEPES (pH 7.4) containing the indicated concentration of trehalose or other sugars. In those cases where the amount

Janoff did not remove extra liposomal hydration buffer as shown below in Col. 10 lines 66-68:

> was obtained as follows: Egg phosphatidylcholine ETVs were prepared as described above using a solute solution (169 mM KGlu, 20 mM HEPES (pH 7.4), 40

In Janoff, extra liposomal hydration buffer was exchanged for sodium buffer as discussed at Col. 11 lines 1-4:

> umol lipid/ml) containing 250 mM trehalose. Subsequently, the external potassium buffer was exchanged for a sodium buffer (150 mM NaCl, 20 mM HEPES (pH 7.4), 250 mM trehalose). Adriamycin (200 nmol/umol

It is well known that NaCl and HEPES buffer gives stable liposomez. On dehydration it is destabilized and they become leaky. Janoff does not show that sucrose alone gives stable liposomes. Janoff shows that addition of sucrose to NaCl and HEPES buffer overcomes this destabilization during dehydration and rehydration.

How does one of ordinary skill in the art know that when sucrose with sodium or potassium buffer and HEPES in hydration medium, which is shown by Janoff to help in protection from leakage of active material from the liposome in which it is entrapped during dehydration and rehydration process, would also help in such protection even when the hydration medium comprises essentially sucrose and ammonium sulphate, and when the dehydration/rehydration is not required? How can one of ordinary skill in the art think that such hydration medium used by Janoff would give long circulation time? How does one of ordinary skill in the art know when sucrose with HEPES and sodium or potassium buffer when used as hydration buffer enabling liposomes to retain ADRIAMYCIN during dehydration/rehydration would increase circulation time ($T_{1/2}$) when sucrose with ammonium sulphate and without HEPES or sodium or potassium buffer is used as hydration medium? It simply is not taught or suggested and hence not obvious. There is no teaching or suggestion in Janoff or any of the cited references, alone or in combination to allow one to come to this conclusion without impermissible hindsight.

In addition, the Examiner's conclusion regarding Janoff when he states: "The phospholipids are dissolved in chloroform, evaporating chloroform and hydrating the phospholipid with a hydrating medium containing the protective sugar and a buffer." There is no such statement in Janoff. Further, such statements are stated out of context to provide support to the presumed argument. The instant claimed invention does not mention that any hydrating medium can be used, but rather has characterized the hydrating medium as consisting essentially of ammonium sulphate and sucrose. Janoff does not use such hydrating medium. At this step, the instant invention does not use any buffer, such as the Hepes buffer used by Janoff. Again in the next line word "buffer" is used to make believe that hydration medium used in the instant invention is same as that used by Janoff. Claim 1 of the instant invention does not say only the quantity of the hydration medium. It first says the hydration media consists essentially of ammonium sulphate and sucrose, and then it states the amount of aqueous hydration media used.

Further, Janoff does not teach the use of 25 ml of hydration medium comprising ammonium sulphate and sucrose per mmole of phospholipid, when he uses 150mM NaCl, 20 mM HEPES (pH 7.4) containing indicated concentration of trehalose or other sugars. As discussed above, there is no ammonium sulphate in Janoff's hydration buffer.

Contrary to the Examiner's assertions, it would have not been obvious to one of skill in the art to include sucrose in the hydrating medium of Slater since Janoff teaches that sucrose enables the liposomes to retain the active agent. First, since Slater relates to a pegylated liposome, one skilled in the art would have no reason to take parts of Slater and apply to a non-pegylated liposome. Further, Slater is not directed to methods of retaining the active material and prolonging circulation but rather to retain the labile topoisomerase inhibitor without opening a lactone by preventing its contact with aqueous phase inside the liposome. With this problem in mind would one skilled in the art look to Janoff's sucrose do that? There is no such indication or suggestion in Janoff. Slater instead uses pegylated phospholipid and makes pegylated liposome to keep the labile therapeutic agent –topoisomerase inhibitor intact.

The Examiner's statement that "the use of histidine as the buffer along with the ammonium sulphate and sucrose would have been obvious to one of ordinary skill in the art since Janoff teaches the inclusion of a buffer and Slater teaches the use of this buffer in the final liposomal preparation" is the Examiner's imagination after reading the instant specification. It is clear hindsight thinking, to compose the present invention by somehow joining fragments from

two different documents, without any connection, one (Slater) is pegylated another (Janoff) is non-pegylated, one is for aqueous contact labile therapeutic agent and the other (Janoff) is for dehydration and rehydration stability of liposomes. Further, the present combination has been suggested to achieve the instant invention, which has the objective of getting liposomes with long circulation time without pegylation. The Examiner has not answered whether such a composition will give a long circulating liposome when mPEG is not used in the phospholipids used i.e. when pegylation is removed and has not provided any evidence that Slater and Janoff teaches or suggests this.

In any event, even combining these references, they do not teach or suggest each and every claim element. As such, applicants request withdrawal of this ground of rejection.

III. Claims 1-8, 10, 12, 14-22 and 63-69 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Forssen (US 5,714, 163) in combination with Janoff (US 4,880,635) and Papahadjopoulos (US 4,235,871), optionally in further combination with Slater (US 6,355,268) and/or Clerc (US 5,939,096).

A. Forssen fails to teach or suggest all of the claimed elements, alone or in combination with Slater (or any other cited art).

Forssen has been discussed in previous responses to office actions and in the Appeal Brief. Forssen does not teach or suggest at least two elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; and 2) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. A detailed analysis follows:

1. Forssen does not teach or suggest the claimed hydration medium

In Example 1 of Forssen, a spray-dried distearoylphosphatidylcholine/cholesterol (DSPC/CHOL) lipid was hydrated with either a buffer containing the ammonium salt of one of the counter-ions or 300 mM sucrose. One of the 10 buffers used contained ammonium sulfate. After sonication, annealing, centrifuging and buffer exchange by gel filtration on a Sephadex column previously equilibrated with unbuffered 300 mM sucrose, vincristine was ion-exchange loaded by incubation of the liposomes with an aqueous solution of its sulfate salt.

Forssen relates to phosphatidyl choline/cholesterol (PC/CHOL) liposomes containing vincristine or other cationic vinca alkaloids and an anion in an aqueous phase of liposome. Reference is made to several prior art methods for forming liposomes and there is no suggestion that Forssen teaches any new or modified method of liposome formation. It is stated that a significant benefit of the liposomes used by Forssen is that they can be prepared without a transmembrane or pH gradient (see column 4, lines 26-30). In the generally exemplified process, PC/CHOL is hydrated with aqueous anion and the vinca alkaloid is loaded by ion-exchange loading (see column 4, line 49 to column 5, line 10).

Column 6, lines 22-25 describe what can be used in the buffer in the Forssen process: "Vesicles were prepared by hydrating approximately 500 mg of sprayed-dried lipids, DSPC:Cholesterol (2:1, mole ratio), at 65°C with either a buffer containing the ammonium salt of one of the counter-ions or 300 mM sucrose" (emphasis added).

Applicants direct the Examiner to Table 1 of Example 1 of Forssen (col. 6, line 53 to col.7 line - 14). When the counter ion is tartrate, the percent of vincristin entrapped is 90% and when the counter ion is sulphate, the percent of vincristin entrapped drops to 49%. This table shows that different counter-ions have different entrapment rates. The Examiner has not explained why one skilled in the art looking at Example 1 of Forssen would choose sulphate as a counter ion especially when there are other ions (except succinate) giving entrapment above 67%. Further, Table 1 has no data for vincristine entrapment when "sucrose" is used (not along with counter ion). Thus, there is no teaching here of a buffer comprising ammonium sulphate and sucrose.

If one reads the same example completely, further down in the animal experiment there is a sentence in column 7, lines 26-32 that reads as follows:

> The mice were randomized into 11 treatment groups and therapy was initiated four days after tumor implantation. The chemotherapeutic treatment groups consisted of free Vcr and nine vesicle-Vcr formulations (Vcr salts of glutamate, tartrate, hydrogen diphosphate, aspartate, EDTA, succinate, pyrophosphate, lactobionate, and citrate). Dosing was at 2.5 mg/kg. Tumor-bearing untreated controls received a treatment of 300 mM sucrose in a volume equivalent to the experimental groups.

Notably from this passage, sulphate is not at all used, which clearly shows that the inventors of Forssen did not think sulphate was useful.

Thus, there is no teaching in Forssen that would have led one of ordinary skill in the art to conclude that the presence of both ammonium sulfate and sucrose in an aqueous medium for hydrating phospholipid would reduce leakage from a loaded liposome. Moreover, there is no reason to conclude from Forssen that sucrose would be required for decreasing leakage or prolonging circulation and/or increasing efficiency in reduction in breast tumor if doxorubicin is loaded by the process of the present invention.

Further, the fact that sucrose or other sugars inhibit leakage of an encapsulated active material from dehydrated liposomes is no indication that it will prevent leakage from the liposomes prior to dehydration or from liposomes that need no dehydration or from the rehydrated liposomes after loading with the required drug or other active material.

2. Forssen does not teach or suggest the claimed sucrose histidine buffer

Moreover, Forssen does not use sucrose-histidine buffer for removing extra liposomal hydration salt. Using a sucrose-histidine buffer for washing extra liposomal hydration salts (ammonium sulphate) is an ingenious technique of getting sucrose in the outside layer. This feature is not an obvious process step, especially in view of the deficient art, and by itself has inventive merit, in achieving removal of hydration media salts and depositing sucrose on the outside layer of the liposome after the liposomes are sized.

There is no reasoning for picking up only ammonium sulphate from 10 different ammonium salts in Forssen and only sucrose from a laundry list of sugars in Janoff. This is only a hindsight thought and is not obvious to a person of ordinary skill in the art.

These deficiencies are not cured by the cited references of Janoff, Papahadjopoulos, Slater, and/or Clerc. As discussed above, Janoff, Papahadjopoulos, and Slater also do not teach or suggest, alone or in combination all of the recited claim elements. Clerc also fails to teach or suggest all of the claimed elements, alone or in combination with Forssen/Slater (or any other cited art).

The Examiner has misread Clerc when he states "while disclosing a therapeutic or diagnostic agent (active material) loading method into the liposomes teaches the hydration of the phospholipids with a solute species which is saline or a disaccharide (sucrose) and a buffer which is the same as the internal or external aqueous medium such as histidine or MES or Tris (Col.7, lines 5-15; col.8 lines 8-15).

First, when referring to the solute species, Clerc is describing external mediums to be used to produce a higher inside/lower outside concentration of the weak acid salt, and is not referring to a hydration medium. Clerc states:

> The external medium preferably contains (i) a buffer, e.g., a 5-50 mM buffer having a pH the same as or similar to the pH of the original hydration medium, and (ii) solute species effective to raise the osmolality of the external medium to close to that of the internal medium, e.g., 200-300 osm/kg. The buffer is preferably one, like histidine, MES or TRIS, which is relatively impermeant, and which exerts maximum buffering capacity in the pH 5-7 range.
>
> The solute species for external-medium osmolality is preferably either the salt of a strong acid, e.g., physiological saline, or a mono- or di-saccharide, such as sucrose, glucose, or mannitol. The latter type of solute is preferred where it is desired to store the liposomes by lyophilization, in which case the saccharide functions as a cryoprotectant to minimize liposome damage during freezing and rehydration.

Col. 8, lines 1-16. Clerc is thus describing a solution used for external-medium osmolality and is not referring a buffer used to remove ammonium sulphate from the extra liposomal hydration medium as required by the claims. Thus, Clerc does not teach or suggest the element of claim 1 of "removing ammonium sulphate from extra liposomal hydration medium by dialysis using a sucrose-histidine buffer solution" as the buffers used in Clerc are instead used to form a liposome pH gradient.

Clerc does not teach or suggest an aqueous hydration media consisting essentially of ammonium sulfate and sucrose and the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes. In describing the hydration medium, Clerc states that the hydration medium is preferably at least 50 mM weak acid salt, which is described in the specification as: a sodium/calcium acetate, sodium/calcium formate, salts of propanoic, butanoic or pentanoic acids. See Col. 7, lines 15-20. There is no teaching of a hydration medium consisting essentially of ammonium sulfate and sucrose and further there is no teaching of using in the range of 10 to 35 ml for each mmole of phospholipid present to form long circulating non-pegylated liposomes, as required by the claims.

Clerc is describing his method as a method of forming liposomes having a higher inside/lower outside pH gradient. The weak acid compounds for loading by his method include ibuprofen, tolmetin, indomethacin, phenylbutazone, meclofenamic acid, piroxycam,

ciprofloxacin and nalidixic acid. There is no reason to look at it for increasing circulating time of liposome without a PEG coating or providing a graft of a hydrophilic polymer chain as mentioned by Clerc. There is no indication that it can be used for therapeutic or diagnostic agents (active material) like doxorubicin. It would not be obvious to one of ordinary skill in the art, to take only from that portion indicated by examiner in Clerc for making the instant invention when the instant invention does not follow a higher outside/lower inside pH gradient system. The instant invention also does not follow providing a coating or a graft of hydrophilic polymer chain to increase the circulating time of liposomes. There is no motivation to take any teaching from Clerc, and no motivation to choose a particular internal/external medium from alternatives suggested in the portion indicated by the Examiner. Imputing motives after reading instant invention is not acceptable, as obviousness decisions should be based on facts existing in the references cited.

Since the cited references do not teach or suggest all of the elements of the claims, alone or in combination, applicants request withdrawal of this ground of rejection.

We do not agree to the examiner's reasoning and conclusions because it is impossible to predict a synergistic property of any composition before actually making it, which would be required if one were to accept the Examiner's rejections. The present invention provides a unique process of making a liposome that resulted in a unique property of increasing half circulation period of the liposome so prepared having at least 25 times longer than that of the conventional non-liposomal compositions.

None of the cited references in any of the rejections teach or suggest, alone or in combination several elements of the claimed invention. It is interesting to note that to argue obviousness of a single claim element, the examiner had to suggest one of its parts from one reference and another part from a different unrelated reference when these parts were used for different purposes to achieve different effects in each of the references. This is clear evidence that the rejections are based on a clear case of impermissible hindsight.

IV. State of the Art

The Examiner has again erroneously asserted that Radhakrishnan and Uchiyama teach hydration of the lipid film with the claimed amounts of hydration medium. This is in error as we

have shown earlier in the Appeal Brief. First Radhakrishnan does not teach or suggest at least two elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; and 2) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution.

Radhakrishnan discloses the use of aerosolized aqueous suspensions of corticosteroid for inhalation to treat respiratory tract conditions or diseases. The exemplified processes for liposome preparation are by solvent-injection (see column 5, lines 5-15 & Example 1A) and lipid film hydration (see column 5, lines 16-29 & Examples 1B & 2). In each case, the corticosteroid is entrapped during liposome formation. There is no disclosure of the presence of ammonium sulfate or sucrose in an aqueous hydration media or of the loading of corticosteroid into a pre-formed liposome.

Radhakrishnan prepares liposomes to entrap lipid soluble corticosteroids, with the object of making them into aerosols for nasal spray, which is not expected to have long circulation requirement. Three processes are described in the examples. In Example 1A – solvent injection method, in Example 1B thin film hydration method and in Example 2 the lipid mixture is lyophilized before hydration. In all these examples corticosteroid Beclomethasone diproprianate (BDP) is taken in the lipid mixture. This procedure is different from the instant invention, wherein the therapeutic agent is loaded in the liposomes after they are formed. In all these three methods, phosphate buffered saline pH 7.4 is used as hydration medium. This is different from the instant invention, which uses ammonium sulphate and sucrose as the hydration medium. There is no step of removing extra liposomal hydration buffer in Radhakrishnan, as there is no step of loading the drug after the liposome is formed as in the instant invention.

Uchiyama also does not teach or suggest at least two elements of the claims: 1) an aqueous hydration medium consisting essentially of ammonium sulfate and sucrose; and 2) removing extra liposomal hydration salt from the liposomal composition using a sucrose-histidine buffer solution. Further, Uchiyama does not teach or suggest "the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid" as required by the claims.

Uchiyama reports the effect of size and fluidity of liposomes on their accumulation in tumors. The liposomes were prepared from EPC or hydrogenated EPC (HEPC), dicetyl phosphate (DCP) and cholesterol (CHOL) in the molar ratio 5:1:4 by hydration with an isotonic

phosphate buffer (not ammonium sulfate and sucrose as required by the present invention). *See* page 196-197. It was concluded that accumulation of liposome from the vascular space into a tumor is primarily governed by their size and not by their membrane fluidity or blood circulation time.

The Examiner's calculation of the amount of aqueous hydration media per mmole phospholipid from Uchiyama (incorrectly) assumes that all lipids are phospholipids. However, 40 mole percent of the lipids is provided by cholesterol (CHOL). Accordingly, the 5 ml phosphate buffer was added to 200 μmole lipid of which 60% was provided by the phospholipids (EPC/HEPC & DCP) and hence the relative amount of hydration media is 41.5 ml per mmole phospholipid. This is outside the range required by the claims.

Regarding Kirpotin, this reference does not also teach or suggest each and every element of the claims alone or in combination with all of the other cited references. Kirpotin was discussed extensively in the Appeal Brief. In addition, the Examiner here cited Example 7 and 8. Example 7 does not have any word on Sucrose. Example 8 has mentioned "if necessary, for addition of dry sucrose to achieve osmolarity of 377 mmole/kg. (These solutions are here after referred to as inner buffers). All solutions additionally contained 10 mM hydroxrethylpiperazino ethane sulphonic acid (HEPES) to stabilize pH at titration end point." Thus, the solution in Kirpotin contains HEPES, where in contrast, the present invention consists essentially of ammonium sulphate and sucrose only.

CONCLUSION

In view of the remarks above, Applicants respectfully submit that this application is in condition for allowance and request favorable action thereon. The Examiner is invited to contact the undersigned if any additional information is required.

Applicants authorize the Commissioner to charge Deposit Account No. 042223, referencing Attorney Docket No. 067042-0005 for any fees due for filing this paper and to credit any overpayments.

Respectfully submitted,

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